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In vitro development of *Encyclea cordigera* in different concentrations of honey

Desenvolvimento *in vitro* de *Encyclea cordigera* em diferentes concentrações de mel

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– NOTE –

ABSTRACT

The objective of this paper was to evaluate the effects of different honey concentrations in culture media, in comparison to sucrose medium, for the in vitro *development of the epiphytic* ***Encyclea cordigera*** *orchid, in order to improve the process of propagation of the species. The* in vitro *germination was prepared on a reduced Murashige & Skoog (MS) medium. After 90 days, the seedlings were divided into different treatments, where they remained for another 90 days. Six treatments were set up (30g L^{-1} of sucrose; 15, 30, 45, and 60g L^{-1} of honey; and absence of any carbohydrates) in a completely randomized design. Plants were removed from the vials 270 days after the start of the experiment, and the number of roots, length of the largest leaf, length of the longest root, number of leaves, and fresh and dry masses were evaluated. Data concerning the number of leaves and roots were $(x+1)^{1/2}$ transformed and subjected to an analysis of variance (ANOVA); the means were compared by a Tukey's test set at 5% probability. Medium containing 60g L^{-1} of honey proved to be superior to the sucrose medium traditionally used, favoring the* in vitro *growth and development of* ***Encyclea cordigera****. This medium can therefore be recommended for the propagation of this species, which is usually cultivated as an ornamental plant.*

Key words: *Brazilian orchid,* in vitro *propagation, carbohydrate source.*

RESUMO

Este trabalho foi realizado com objetivo de avaliar a influência da concentração de mel no meio de cultura, comparando-o com a sacarose, para o desenvolvimento in vitro *da orquídea epífita* ***Encyclea cordigera****, a fim de aperfeiçoar o processo de propagação dessa espécie. A semeadura* in vitro *procedeu-se em meio de cultura Murashige & Skoog (MS) reduzido e, após 90 dias, as plântulas foram distribuídas entre os tratamentos, em que permaneceram por mais 90 dias. Seis tratamentos (30g L^{-1} de sacarose, 15, 30, 45 e 60g L^{-1} de mel e ausência de carboidrato) foram utilizados, em delineamento inteiramente casualizado. Após 270 dias do início do experimento, as plantas foram retiradas dos frascos, sendo avaliado o número de raízes, comprimento da maior folha, comprimento da maior raiz, número de folhas, massa fresca e massa seca. Os dados de número de folhas e de raízes foram transformados $(x+1)^{1/2}$ e submetidos à análise de variância (ANAVA); as médias foram comparadas pelo teste de Tukey a 5% de probabilidade. O meio de cultura suplementado com 60g L^{-1} de mel favoreceu o crescimento e desenvolvimento* in vitro *de* ***Encyclea cordigera****, sendo superior ao uso de sacarose, que é tradicionalmente usado, podendo ser recomendado para propagação dessa orquídea, que apresenta potencial ornamental.*

Palavras-chave: *orquídea brasileira, propagação* in vitro*, fonte de carboidrato.*

The ***Orchidaceae*** is one of the largest angiosperms families, comprising approximately 780 genera and 25,000 species (PRIDGEON et al., 2009). Genus ***Encyclea*** is widely dispersed, ranging from Florida and Mexico to South America, where several are native from Brazil (WATANABE & MORIMOTO, 2007).

The orchid's germination can occur as a result of a symbiotic relationship with mycorrhizal fungi, or asymbiotically, *in vitro*, using an aseptic nutrient medium containing mineral salts and a carbon source (CAMPOS, 1996). *In vitro* fertilization is an indispensable tool for the mass propagation of the main species of orchids (SANTOS et al., 2006).

According to NEPOMUCENO et al. (2009), seedlings grown *in vitro* may not have suitable lighting conditions and CO_2 concentrations, and sometimes do not have sufficient chlorophyll levels for photosynthesis to sustain growth. Therefore, the growth of most cultures is sustained by a source(s) of carbohydrate(s) added to the medium. Carbohydrates provide both metabolic energy and carbon skeletons necessary for the biosynthesis of amino acids and proteins, as well as structural polysaccharides such as cellulose, that is, all the organic compounds required for cell growth (CALDAS et al., 1998).

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Exogenous carbon in plant tissue cultures serves as an energy source, influencing the plant's physiology and differentiation of organs, and the type and concentration of the sugars are important to promote *in vitro* germination and growth (MOREIRA et al., 2007).

Sucrose is the most commonly used carbohydrate in culture media, supporting the highest growth rates for most species (CALDAS et al., 1998). However, there are alternative carbohydrate sources, such as honey, that have never been tested in the *in vitro* cultivation of plants.

Honey is a sweet, viscous substance, consisting essentially of different sugars, particularly glucose and fructose, accounting for about 70% of the total carbohydrates (MOREIRA & DE MARIA, 2001). In addition to sugars, honey contains enzymes, vitamins, amino acids, minerals, bactericides and aromatic substances, organic acids, phenolic acids, flavonoids, pollen, and bees wax (KOMATSU et al., 2002).

The objective of this study was to evaluate the effects of different doses of honey (obtained from the sugarcane sap of reed beds) produced by the honey bee ***Apis mellifera***, relative to the carbon source commonly used in the *in vitro* cultivation of orchids, in order to improve the *in vitro* production of orchid seedlings, such as those of ***Encyclea cordigera***, which has important ornamental and ecological value.

Sealed capsules containing mature ***Encyclea cordigera*** seeds were sterilized in 70% ethanol for 5 minutes followed by sodium hypochlorite with 1% active chlorine for 30 minutes, rinsed three times with distilled water, autoclaved, and then opened inside a laminar flow cabinet. Approximately 100mg of seeds was inoculated in 500ml transparent plastic bottles containing 40ml of the reduced Murashige & Skoog (MS) nutrient medium that had been autoclaved at 121°C and 1.1atm for 15min (CALDAS et al., 1998).

The reduced MS medium contains half the concentration of salts and macronutrients and the same micronutrient concentration as that developed and proposed by MURASHIGE & SKOOG (1962), supplemented with vitamins, inositol, and glycine (COSTA et al., 2009), 2% sucrose, 5.7 adjusted pH, and gelled with 0.7% agar. Germination and growth took place in an incubation room with a temperature of 25±2°C and a 16-hour photoperiod at approximately 75µmol.$\cdot m^{-2}$.

After 90 days, seedlings measuring 1.0±0.3cm were transferred onto the reduced MS medium containing 0.7% agar and a 5.7 adjusted pH, in which the six treatments (30g L^{-1} sucrose; 15, 30, 45 and 60g L^{-1} honey; and an absence of any carbohydrates) were autoclaved at 121°C and 1.1atm for 15min and distributed in a completely randomized design. Five replicates containing 12 plants were used in each treatment, totaling 360 seedlings.

Seedlings were picked again at 90-day intervals for their respective treatment. Plants were evaluated 270 days after sowing. The following parameters were evaluated: number of leaves (NL), number of roots (NR), length of the largest leaf (LLL), length of the largest root (LLR), fresh mass (FM), and dry mass (DM). The data concerning the number of leaves and roots were $(x+1)^{1/2}$ transformed and subjected to an analysis of variance (ANOVA). The means were compared by a Tukey's test set at 5% probability with the help of the statistical analysis program SISVAR 4.3 (FERREIRA, 2008).

The growth of ***Encyclea cordigera*** seedlings was influenced by the absence or presence of honey in the culture medium, indicating that honey can affect the physiology and *in vitro* growth of orchids.

The best average results for root formation and extension as well as for seedlings with well-developed shoots, and hence more mass (Table 1), were obtained with the addition of 60g $L^{-1}$of honey to the culture medium.

The supply of exogenous sugar can increase the starch and sucrose reserves in micropropagated plants, favoring their acclimatization and accelerating their physiological adaptations (HAZARIKA, 2003). Although honey has never been used as a culture medium additive for the *in vitro* growth of orchids, it has great potential due to the presence of reducing sugars in its composition (Table 2).

Table 1 - Mean values for the number of leaves (NL), length of the largest leaf (LLL) in mm, number of roots (NR), length of the largest root (LLR) in mm, total fresh mass (FM) in mg, and dry mass (DM) in mg of ***Encyclea cordigera*** seedlings grown *in vitro* in different concentrations of honey.

Treatments	NL	NR	LLL mm	LLR mm	FM mg	DM mg
30g L^{-1} sucrose	2.47abc	2.62c	62.80c	27.25cd	338.70c	13.55c
15g L^{-1} honey	2.53a	2.26d	51.05c	21.90d	331.15c	12.25c
30g L^{-1} honey	2.50ab	3.02b	113.90b	48.45b	740.15b	29.50b
45g L^{-1} honey	2.45bc	2.71c	52.85c	31.75c	364.00c	14.55c
60g L^{-1} honey	2.41c	3.61a	138.40a	63.70a	1055.05a	42.30a
no carbohydrates	2.31d	1.98d	28.35d	14.55e	159.22c	5.80c
CV %	1.32	4.77	10.25	9.00	19.48	19.83

Mean values followed by the same letters in the same column do not differ among themselves by a Tukey's test at a 5% significance level.

Table 2 - Results of the analyses of the honey used in the *in vitro* cultivation of ***Encyclea cordigera*** according to the method proposed by MATISSEK et al. (1998).

Variable	Values (%)	Normal values (%)
Sucrose	0.48	0.2–11.4
Reducing Sugars (RS)	84.8	59–76
Acidity	0.48	<0.245

The culture medium supplemented with 60g L^{-1} of honey favored the *in vitro* growth and development of ***Encyclea cordigera*** seedlings, and therefore, it can be recommended for the propagation of this specie, which has the potential for ornamental use.

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